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The emergence and cross species transmission of newly discovered tick-borne Bunyavirus in China

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A novel tick-borne Bunyavirus, discovered in China and later in South Korea and Japan, is now known as Huaiyangshan virus or severe fever with thrombocytopenia syndrome virus and has been identified as the causative agent of a hemorrhagic fever-like disease. Of five species of ticks carrying Huaiyangshan viruses, *Haemaphysalis longicornis* was the most abundant in regions where the virus was endemic. Its usual hosts (cattle, goats, dogs, rats and chickens) tested positive for Huaiyangshan virus RNA and had a high seroprevalence. The distribution of *H. longicornis* and the migratory routes of four wild fowl across China, South Korea and Japan are coincident. Thus a tick and migratory bird model for the transmission of the Huaiyangshan virus was proposed.

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Introduction

In 2009, a novel hemorrhagic fever-like disease was first observed in China [1] and a novel Bunyavirus was identified as the causative agent in 2010 [1]. Surveillance and epidemiologic investigations confirmed its wide geographic distribution in China [2]. From 2010 to 2013, 2047 cases of human infections were reported, and the virus was found to be present (designated as endemic) in 16 provinces in the central and eastern regions in China [3]. 94.4% of these cases were farmers or forest workers, who lived in rural areas and engaged in the agricultural activities [3,4]. Subsequently, the disease was identified in South Korea and Japan in 2012 [5–7]. Retrospective studies dated the earliest known human infections back to 2005 in Japan and 2006 in China [6,8]. The broad geographic distribution of the virus across China, Korea and Japan, its high fatality rate, and the potential for human-to-human transmission led this virus to be seen as an increasingly important threat to regional and global health [9].

Emergence of the new virus

In April 2009, physicians of the Union Hospital of Tongji Medical College, Wuhan, China noticed several fatalities among a cluster of patients with high fever, leucopenia, thrombocytopenia, and elevated liver transaminase levels [1,10,11•]. All patients were from the Huaiyangshan mountain area of Hubei and Henan provinces, in the central part of China. Some of the patients recalled a history of tick bites [1,10,11•]. As the clinical features were highly similar to those of human granulocytic anaplasmosis (HGA), caused by the bacterium *Anaplasma phagocytophilum* and first reported in the neighboring province of Anhui in 2006 [12], the illnesses was clinically diagnosed as suspected HGA in 2010 [1,10,11•]. Samples from patients were sent to the National Institute for Communicable Disease Control and Prevention of the China CDC for confirmation of HGA infection. HGA was immediately excluded as the cause since all specimens tested negative for *A. phagocytophilum* by PCR and immunologic methods [12]. Fortunately, attempts to isolate the etiologic agent by culturing in DH82 cells were finally successful, leading to the discovery of a novel Bunyavirus, which was published in March 2011 in Chinese Journal of Epidemiology [1].

As several groups were studying the disease and its agent in the initial phase, it was reported as Huaiyangshan hemorrhagic fever (HYSHF) [1], severe fever with thrombocytopenia syndrome (SFTS) [2], and fever, thrombocytopenia and leukopenia syndrome (FTLS) [13]. Accordingly, the etiologic agent was named as Huaiyangshan virus (HYSV), severe fever with thrombocytopenia syndrome virus (SFTSV) and Henan fever virus (HFV). As the geographic origin of the initially reported patients was the Huaiyangshan mountain area and severe fever and thrombocytopenia are not specific for any given disease, but present in nearly all hemorrhagic fevers caused by other viruses, we recommend that the disease be referred to as Huaiyangshan hemorrhagic fever (HYSHF) and the causative virus as Huaiyangshan virus (HYSV) [1,14]. These were the names used in the first published report on this disease and virus, although it appeared in a Chinese language journal [1], and are used in this review. To date, human infections with HYSV

have also been reported in South Korea and Japan [5,6,15].

Genetic characteristic of HYSV

Huaiyangshan virus belongs to the genus *Phlebovirus* in the family *Bunyaviridae* and has been isolated successfully from human and animal samples in China. Like other members of the *Bunyaviridae*, HYSV is an enveloped, negative-stranded RNA virus [1]. Its genome consists of three RNA segments: small (S), medium (M), and large (L). The L segment has a length of 6, 368 nucleotides, with an 11 nucleotide 5′-noncoding region (NCR), an open reading frame (ORF) encoding the RNA-dependant RNA polymerase (RdRp) of 2084 amino acids, and a 113 nucleotide 3′-NCR [16]. The M segment has a length of 3378 nucleotides, with an 18-nuclotide 5′-NCR, an ORF coding for a Glycoprotein N and C (GnGc) precursor of 1073 amino acids, and a 138-nucleotide-long 3′-NCR. Similar to the Uukuniemi-like viruses, HYSV does not have the non-structural protein found in the Phlebotomus fever viruses, however its two surface glycoproteins are in the same order (Gn-Gc) as both these groups. The S segment has a length of 1746 nucleotides, with a 43-nucleotide 5′-NCR, an ORF coding for an N protein, an ORF coding in the opposite sense for an NSs protein and a 29-nucleotide 3′-NCR. A typical characteristic of *Bunyaviridae* family viruses is that the 3′ and 5′ ends of each RNA segment are conserved and form stable base-paired panhandles. In HYSV, the L and M segments have one nucleotide different from the consensus at the 5′ end.

The HYSVs from China, South Korea, and Japan are closely related to each other [16,17••,18,19••] with a nucleotide divergence of less than 6% for all three segments. To date, all known HYSVs identified in China can be classified into six lineages [14,16,18] and there are three lineages of HYSVs in Japan [19••] with the third lineage from Japan closely related to the HYSVs from Zhejiang Province, China. These data suggest that these viruses have a recent common ancestor, likely due to migration of their hosts. Genetic recombination and reassortment between different HYSVs might have occurred [20,21].

Clinical presentations of HYSHF are nonspecific

The clinical presentations of HYSHF are nonspecific, and are difficult to distinguish from those of HGA, hemorrhagic fever with renal syndrome (HFRS), and leptospirosis. The first group of patients presenting to the Union Hospital of Tongji Medical College, Hubei Province in 2009, were initially suspected to have HGA, before a diagnosis of HYSHF was made [1].

Haemaphysalis longicornis ticks are the major vector

Investigation of the first 33 laboratory-confirmed cases revealed that all patients were farmers, living in villages along the low Huaiyangshan mountain area, and involved in domestic animal herding, tea collection and other agricultural activities [2]. From April to May 2010, 613 adult ticks were collected from domestic animals around the villages where these patients lived and nearby regions. Only two species were identified; *H. longicornis* (88.09%) and *Boophilus microplus* (11.91%). The HYSV virus tested positive in the *H. longicornis* (4.93%) and *R. microplus* (0.613%) tick pools. The HYSV-positive *H. longicornis* ticks were only found in the endemic regions (where patients had been infected with HYSV), whereas HYSV-positive *R. microplus* ticks were found in both endemic and nonendemic regions. On the basis of this observation, we hypothesized that *H. longicornis* was the major vector of HYSV [1].

To test this hypothesis, we extended the sampling sites to 15 endemic counties of Hubei and Henan provinces, and 23 non-endemic counties of Hubei Province, around the Huaiyangshan area. In the 15 endemic counties, *H. longicornis* ticks ($N = 3498$) were more abundant than *R. microplus* ticks ($N = 1003$). In the 23 nonendemic counties, *R. microplus* ($N = 12\,830$) was more abundant than *H. longicornis* ($N = 400$) [16]. As in the smaller study [1], the HYSV-positive *H. longicornis* ticks were found only in the endemic counties while HYSV-positive *R. microplus* ticks were found in both endemic and non-endemic counties. These observations supported the hypothesis that *H. longicornis* is the major vector of HYSV [16].

A similar situation was found in Shangdong Province of China, where infections have been identified since 2010. Wang *et al.* reported that 96.9% ticks ($N = 3145$) collected were *H. longicornis*, followed by *R. microplus* (2.6%), *H. campanulata* (0.3%) and *Dermacentor sinicus* (0.2%). HYSV was only detected in *H. longicornis* (96.3%) and adult *R. microplus* (3.7%) [22•]. Recently, Meng *et al.* detected HYSV in 4.17% ($n = 72$) of *H. concinna* ticks from goats [23].

In South Korea, Park *et al.* found that 90.8% of ticks ($N = 13\,053$) collected from 2011 to 2012 were *H. longicornis*, followed by *H. flava* (8.8%), *Ixodes nipponensis* (0.3%) and *I. persulcatus* (0.05%). The HYSV-positive ticks were widely distributed in the country and an HYSV infection was confirmed in 2012 in Gangwon Province where virus-positive ticks had been previously detected [5,24].

Yun *et al.* collected 261 ticks from humans during May–October 2013 in South Korea. They found that the most abundant tick from humans was *H. longicornis* (81.2%), followed by *Amblyomma testudinarium* (6.5%); *I. nipponensis* (5.7%); *H. flava* (5.4%), *H. japonica*, *I. persulcatus*, and *I. granulatus* (0.4% each). The minimum infection rate per 100 ticks (MIR) was 5.7% in *H. longicornis*, 23.5% in *A. testudinarium* and 13.3% in *I. nipponensis* ticks [25••]. This finding of other species of ticks harboring HYSV should

be further addressed. However, it is clear that *H. longicornis* ticks are the major species in the environment of endemic HYSV areas and are the major vector for HYSV [5,16,25••,26].

Domestic animals are widely infected

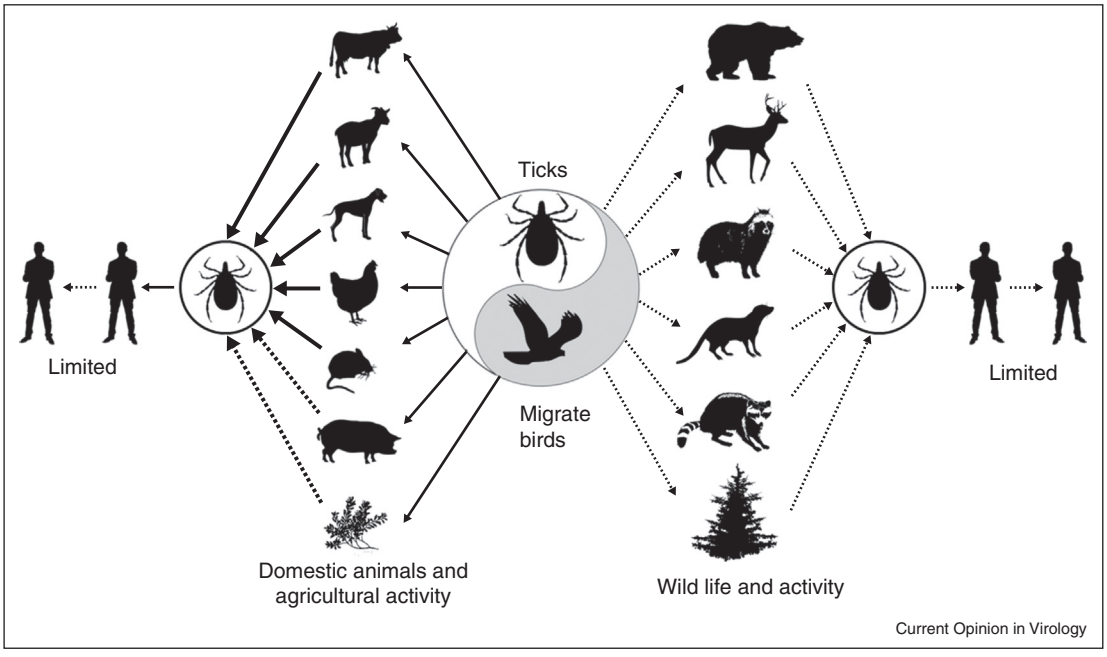
Zhang *et al.* found that some ticks from cattle, goats, dogs and cats in the Huaiyangshan mountain areas tested positive for HYSV [1,16]. Wang *et al.* reported that the prevalence of HYSV-positive ticks from sheep and dogs was higher than that in ticks from cattle in Shandong Province [22•]. Ding *et al.* noted that the seroprevalence for HYSV was 41.8% (268/641) in domestic animals in the HYSV endemic region of Laizhou City, Shandong Province; specifically, goats (74.8%), cattle (57.1%), dogs (52.1%), chickens (35.9%) and pigs (0%) [27]. Niu *et al.* sampled 3039 animals in two endemic counties of Shandong Province from April to November 2011, and found that seroprevalence for HYSV was 69.5% for sheep, 60.5% for cattle, 37.9% for dogs, 3.1% for pigs, and 47.4% for chickens [28]. Li *et al.* found that in six endemic counties of Jiangsu Province from March 2012 to February 2013, the seroprevalence in goats, cattle, dogs, pigs, chickens, geese, rodents and hedgehogs was 66.8%, 28.2%, 7.4%, 4.7%, 1.2%, 1.7%, 4.4% and 2.7%, respectively [29•]. In all these cases serum samples were tested by double-antigen sandwich ELISA. Using the same method, Xing *et al.* found that seroprevalence ranged from 10% to 18% in samples from cattle, sheep, goats, deer, and elk in 24 Minnesota counties of the USA [30]. A possible cross-reaction with Heartland virus, which is phylogenetically related to HYSV and extensively identified in USA, should be considered when interpreting this result [31].

HYSV RNA has also been detected in some animals. Ni *et al.* investigated rodents as possible hosts of this virus and captured 40 *Rattus norvegicus* in villages near the houses of patients as well as eight *Apodemus agrarius* and four *Rattus losea* in the mountainous regions near villages where human cases of HYSHF had been reported in Zhejiang Province from 2012 to 2014. Two of the eight *A. agrarius* rats were positive for HYSV RNA and virus was isolated from the lung tissues of the two animals. The genomic sequences of the rat viruses had a high level of sequence identity to those isolated from patients [32•]. Although seropositive rodents have also been reported, more study is required to clarify if rodents have a role in the transmission of the virus [29•]. Niu *et al.* reported that HYSV RNA was detected in all sampled animal species, ranging from 1.7% to 5.3%, and viruses were isolated from sheep, cattle, and dogs [28]. Again, the role played by these animals in transmission of the virus needs further study.

A tick and migratory bird model for HYSV transmission

Using whole genome sequences, Yun *et al.* analyzed the phylogenetic relationships among HYSVs and showed

Figure 1



Tick and migratory bird pathway for the transmission of HYSV. The thicker solid and dashed arrows from domestic animals and agricultural activity indicate that virus was isolated or viral RNA was detected, respectively. The dashed arrows in wildlife and activity indicate viral RNA was detected in ticks captured from those wild animals and grassland.

that it had been circulating between China, South Korea, and Japan and that HYSV-infected *H. longicornis* and human infections had been found in all three countries. They hypothesized that HYSV was transmitted, at least in part, by the *H. longicornis* tick and moved over long distances to Korea and Japan through migratory birds, especially across water bodies of the East China Sea, Yellow Sea, and the East Sea/Sea of Japan [33••].

This hypothesis is supported by the following observations. First, *H. longicornis* is the most abundant of the HYSV-positive ticks in the endemic regions, and widely distributed in the Asia-Pacific region, including China, Korea, Japan, Australia, the Pacific Islands, and New Zealand [34–38]. Second, four migratory wild fowl species that are regular hosts of *H. longicornis* (*Zoothera aurea*, *Turdus hortulorum*, *Halcyon coromanda*, and *Pitta nympha*) are known to breed and migrate between China, South Korea, and Japan [39–42]. Third, the tick distribution matches the migratory bird flyways between China, South Korea, and Japan [43•,44]. Jeju Island, Korea, lying on the East Asia/Australasia Flyway, is home to one of the largest populations of migratory birds in Korea, and the place where 10 of 36 HYSV cases were identified [33••].

Taking the above observations together, a tick and migratory bird model for HYSV transmission is proposed here (Figure 1). Human infections by ticks from domestic animals and agricultural activities have been extensively recorded. HYSV has been isolated from ticks of cattle, goats, dogs and rats. Virus RNA has been detected in ticks from pigs and tea bushes, and domestic animals in endemic regions have high seroprevalence. The ticks of *H. longicornis* and *I. nipponensis* have been frequently found on wild boar, roe deer, raccoon dogs, Siberian weasels, Asian badgers, and leopard cats [24,45]. However, while the infection of humans by virus positive ticks from wildlife is very likely, more evidence is needed for the case of this specific pathogen. The following questions remain to be answered: where did the virus positive ticks from domestic animals originate, and how do migratory birds distribute virus positive ticks? This model needs to be further tested, but it may provide new strategies for the prevention and control of HYSHF.

Conclusion

In 2009, a cluster of patients, from the Huaiyangshan mountain areas on the border of Hubei and Henan provinces of China, who had high fever, leucopenia, thrombocytopenia and elevation of liver transaminase levels, was first noticed at the Union Hospital of Tongji Medical College. A novel tick-borne Bunyavirus was identified as the causative agent. It was first named as HYSV, and later named as SFTSV. The disease is a hemorrhagic fever-like disease, and is widely distributed in China, South Korea and Japan. The clinical presentations of severe fever and thrombocytopenia are consistent with many bacterial or virus infections. Of the five species of ticks positive for Huaiyangshan virus RNA, *H. longicornis* was the most abundant in endemic regions, and is considered to be the major vector. Its main hosts in endemic regions include cattle, goats, dogs, chickens, and rats, which were widely infected wit HYSV as evidenced by various levels of seroprevalence. The *H. longicornis* ticks were hypothesized to play a central role in transmitting the virus, at least in part, by being transported over long distances to Korea and Japan by migratory birds. On the basis of the above observations, a tick and migratory bird model for HYSV transmission was proposed.

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